were determined by semi quantitative RT-PCR. In two patients, a biopsy of the repair tissue was taken 6 and 18 months after ACI.
Results: In total, $0.81\pm0.33\times10^6$ cells per gram tissue could be recovered with no difference between dissecate and notch cartilages (cell viability $\geq$ 90%). Compared with the notch chondrocytes, cells from the dissecate expressed similar levels of collagen type I and -II mRNA including a 100,000fold relative increase of col1 over col2 after cell expansion. Expression of collagen type X mRNA is significantly less in trauma joints compared to OCD cartilages before and after cell culture. The level of collagen type X message is approx. 50fold decreased after cell culture, indicating a loss of hypertrophic cells or expression of hypertrophic genes in chondrocytes. Post-implantation biopsies show features of hyaline-like cartilage without signs of hypertrophy or mineralization.
Conclusions: The high viability, quality and activity of the extracted cells suggest a still preserved intrinsic repair capacity of the dissecates. The molecular analysis indicates phenotypic modulation of the isolated chondrocytes during cell culture. The similar quality of the cells from both dissecate and notch after cell culture suggests the use of either cartilages as a cell source for ACI.

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bFGF INCREASES Hes1 AND Hey1 EXPRESSIONS IN MURINE CHONDROCYTES IN VITRO

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Purpose: During osteoarthritis (OA), articular chondrocytes undergo cellular changes, such as proliferation and dedifferenciation, reminiscent of growth plate (GP) terminal différentiation. Among the factors and the signaling molecules which control this process during development, some of them may be involved in the pathogenesis of adult cartilage degradation. Basic FGF (bFGF) is a well-known mitogenic factor and it is found at increased concentrations in synovial fluid from OA patients. Recently, it has become evident that the Notch signaling family contributes to chondrogenesis. After activation by extracellular proteolytic cleavages, the intracellular fragment of Notch receptors activates Hes and Hey transcriptional factors. Notch1 has been proposed to be involved in OA degradative process but the role played by Hes/Hey in chondrocytes remains elusive.
The purpose of this study was to investigate the effects of bFGF on the Notch1/Hes/Hey pathway in chondrocytes in vitro.
Methods: Murine chondrocytes were established in primary culture and treated or not with bFGF (1-10 ng/mL) for different periods of time. Notch1, Hes1, Hey1 localizations were analyzed by immunocytochemistry and their expression was analyzed by qPCR and western blot. Target genes were determined by using specific siRNA.
Results: Notch 1 was expressed and activated in bFGF-treated as well as in non treated control chondrocytes. Hes1 mRNA expression was stimulated in a rapid and robust way by bFGF (8-fold higher than in controls after 80 mn exposure). This stimulation was followed by a progressive decrease, although Hes1 expression stayed more elevated than in controls until 24h. Hey1 mRNA expression was also transiently stimulated by bFGF, with a lower maximum level and slightly later than that found on Hes1 expression (5-fold higher than in controls after 3h exposure). The use of specific inhibitors showed that bFGF-induced stimulation of Hes1 or Hey1 mRNAs were transcriptional, and independent of the canonic Notch/Hes pathway. However, while the stimulating effect of bFGF was direct on Hes1 transcripts, its effect on Hey1 mRNA required de novo protein synthesis. The use of anti-Hes1 siRNA strongly suggested that bFGF-induced Hey1 stimulation is under the control of Hes1.
Conclusions: Hes1 and Hey1 are novel target genes of bFGF in murine chondrocytes in vitro and do not involve de novo activation of Notch1 signaling pathway. The bFGF-induced stimulation of Hey1 is dependant of Hes1. Further works are required to determine whether Hes1/Hey1 mediate some of the bFGF effects on chondrocytes.

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EXPRESSION OF THE TYPE IID PROCOLLAGEN ALTERNATIVE TRANSCRIPT DURING ATDC5 CELL CHONDROGENESIS IN VITRO

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Purpose: It has been shown that a transition occurs between the expression of the exon 2-containing IIA form and the exon 2-lacking IIB form of Col2a1 mRNA during chondrogenic differentiation. We have previously demonstrated that this process is more complex than previously believed, and that two additional Col2a1 alternative splice products, termed IIC and IID are expressed in differentiating rabbit and human mesenchymal stem cell (MSC) pellet cultures. The murine embryonal carcinoma-derived cell line ATDC5 has been used as a model of chondrogenesis in numerous recent investigations of skeletal development. We have used this model system to investigate the expression of the known IIA and IIB splice products, and the novel IIC and IID splice products of Col2a1 mRNA during differentiation over 21 days of culture.
Methods: ATDC5 cells were plated at 1.2×10^5 cells/35 mm dish in DMEM: Ham's F-12 (1:1) containing 5% FBS, 10 μg/ml human transferrin and 3×10^{-8} M sodium selenite. After 7 days of culture, the medium was supplemented with 10 μg/ml bovine insulin to induce chondrogenesis over the following 21 days. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air for the entire culture period, and medium was changed every two days. Total RNA was purified from ATDC5 cells with the Nucleospin RNA II kit (Clontech). Synthesis of cDNA was performed using the High Capacity cDNA Reverse Transcription Kit (ABI). Custom Taqman assays (ABI) were designed to detect each splice form of Col2a1 mRNA by placing PCR primers on either side of each splice site, and probe oligonucleotides across the exon-exon junction. A predesigned Col2a1 assay was ordered from ABI to measure total Col2a1 mRNA. Relative quantification was performed using an ABI 7500 Real-Time PCR System.
Results: Col2a1 expression was detectible over the entire culture period, increasing dramatically from day 6 to 21. The relative expression profile for the Col2a1-IIB splice form was not identical to the total Col2a1 profile, indicating the contribution of additional splice forms to the total Col2a1 levels. As in previously analyzed MSC cultures, the IIC form was not detectible in this assay. The Col2a1-IIA splice form was detectible throughout the time course, increasing in abundance with time, but remaining relatively constant from day 14 to 21, whereas the IIB form increase approximately 4-fold over this period. We observed that a significant portion of exon 2-containing procollagen II mRNA, previously assumed to be a single "IIA" isoform is alternatively spliced to contain an additional 3 nucleotides at the 3'-end of exon 2, to yield an additional "IID" isoform.
Conclusions: The IIB splice form, in which exon 1 is directly spliced to exon 3 of type II procollagen mRNA, is made by mature chondrocytes, and is the predominant form in the cartilage extracellular matrix. The IIA alternate splice form contains exon 2 spliced between exons 1 and 3, coding for an additional 69 amino acid cysteine-rich domain in the NH2-propeptide, and is produced mainly by chondroprogenitor cells in the mesenchyme and perichondrium. IIA procollagen is thought to function to bind and control the distribution of TGFβ and BMP-2 in the cartilage ECM. We have previously described an additional splice form termed IID expressed in rabbit and human MSCs, containing an additional amino acid (Trp in human, and Arg in rabbit) at the C-terminal end of the cysteine-rich domain. We have shown in the present study that IID procollagen mRNA is produced by ATDC5 cells. If translated, the IID form may have a function distinct from that of IIA procollagen in the ECM. Previous studies that have detected IIA procollagen mRNA in developmental systems may actually have measured the sum of IIA and IID procollagen, and should be reexamined using methods able to detect the additional IID-specific codon.

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EVIDENCE FOR A NOVEL DESTRUCTIVE MECHANISM IN CARTILAGE INVOLVING EXTRUSION OF MINERALISED MATRIX FROM THE ARTICULAR CALCIFIED CARTILAGE AND SUBCHONDRAL BONE

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Purpose: Osteochondral injury is common in athletes, and is extremely